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(54) **NEW TH17 DIFFERENTIATION MARKERS FOR ACNE AND USES THEREOF**

NEUE TH17-DIFFERENZIERUNGSMARKER FÜR AKNE UND IHRE VERWENDUNG

NOUVEAUX MARQUEURS DE DIFFÉRENCIATION DES TH17 POUR L'ACNÉ ET LEURS UTILISATIONS

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Description

[0001] The disclosure is related to a novel characterization process of acne by identifying for the first time in the inflammatory process the involvement of Th17 cells, and to the therapeutic applications targeting the function of Th17 cells in acne.

[0002] More specifically, the disclosure provides the use of ROR gamma t (also known as RAR-related orphan receptor C or retinoic acid -related orphan receptor (ROR)gamma]t or ROR C variant 2 or RORγ2)), or ROR alpha (also called RORA or RAR-related orphan receptor A), and their use to diagnose acne and/or to screen inhibitors of Th17 differentiation, notably in inhibiting ROR gamma t or ROR alpha and the use of these screened inhibitors in acne treatment.

[0003] Acne is the most common skin condition affecting millions of people worldwide. Patients with severe acne frequently face significant psychological and emotional problems due to the scarring associated with the disease. The pathogenesis of acne vulgaris is complex and incompletely understood.

[0004] Inflammation is one of the key components of the pathogenesis of acne. An immunological reaction to the gram-positive microbe P. acnes may play a major role in the initiation of the inflammatory reaction (De Young LM, Young JM, Ballaron SJ, Spires DA, Puhvel SM. Intradermal injection of Propionibacterium acnes: a model of inflammation relevant to acne. J Invest Dermatol. 1984 Nov;83(5):394-8, Jappe U, Ingham E, Henwood J, Holland KT. Propionibacterium acnes and inflammation in acne; P. acnes has T-cell mitogenic activity. Br J Dermatol. 2002 Feb;146(2):202-9). Recently published studies also implicate Toll Like receptor 2 (TLR-2) in inflammatory acne (Fathy A, Mohamed RW, Ismael NA, El-Akhras MA. Expression of toll-like receptor 2 on peripheral blood monocytes of patients with inflammatory and noninflammatory acne vulgaris. Egypt J Immunol. 2009;16(1):127-34; Nagy I, Pivarcsi A, Koreck A, Széll M, Urban E, Kemény L. Distinct strains of Propionibacterium acnes induce selective human beta-defensin-2 and interleukin-8 expression in human keratinocytes through toll-like receptors. J Invest Dermatol.2005 May;124(5):931-8).

[0005] Recent reports demonstrate that the skin expresses various antimicrobial peptides in response to the proliferation of pathogens as part of cutaneous innate immunity (Braff MH, Bardan A, Nizet V, Gallo RL. Cutaneous defense mechanisms by antimicrobial peptides. J Invest Dermatol. 2005 Jul;125(1):9-13; Schröder JM. Epithelial antimicrobial peptides: local innate defense effector molecules]. Ann Dermatol Venereol. 2004 Apr;131(4):411-6; Selsted ME, Ouellette AJ. Mammalian defensins in the antimicrobial immune response. Nat Immunol. 2005 Jun;6(6):551-7). Important amongst this group of antimicrobial agents include members of the human β defensin family and granulysin-derived peptides (Deng et al, 2005; Harder et al, 2004; McInturff et al, 2005). Human β defensin-1 and 2 (HBD-1 and HBD-2) are expressed in the pilosebaceous unit and their expression is upregulated in acne lesions (Chronnell et al, 2001). Recent studies have also discovered that select strains of P. acnes can activate HBD-2 through TLRs further confirming the importance of these peptides in inflammatory acne (Nagy I, Pivarcsi A, Koreck A, Széll M, Urban E, Kemény L. Distinct strains of Propionibacterium acnes induce selective human beta-defensin-2 and interleukin-8 expression in human keratinocytes through toll-like receptors. J Invest Dermatol.2005 May;124(5):931-8).

[0006] For the first time, the applicant proposes with experimental evidences to target a novel inflammatory process, the Th-17 cells differentiation for treating and/or diagnosing acne.

[0007] Thus, the disclosure is relating to the use of the mRNA encoding ROR gamma t, and also the corresponding proteins, as markers for acne; as well as the use of the mRNA encoding ROR alpha, and also the corresponding proteins, as markers for acne. The invention is also relating to the use of at least one of the proposed markers of the invention and/or at least one of the markers chosen from IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-23 A, IL-26, CCL20, as markers for acne. The disclosure provides a method for the diagnosis of acne, comprising the following steps:

a) detecting the level of expression of at least one of the proposed markers of the invention (ROR gamma t or ROR alpha), and/or at least one of the markers chosen from IL-17A, IL-17F, IL-22, CCL20 in a sample taken from an individual,
b) detecting the level of expression of at least one of the proposed markers of the invention, and/or at least one of the markers chosen from IL-17A, IL-17F, IL-22, CCL20 in a sample taken from a healthy individual,
c) comparing the difference in level of expression of at least one marker and for which the level of expression is significantly higher in the individual than the level of expression in the healthy individual;
d) the overexpression of at least one of the markers of step c) being an indicator of acne, thus diagnosing acne.

[0008] The disclosure provides also a method for the diagnosis of acne that can also comprise the following steps:

a) detecting the level of expression of at least one of the proposed markers of the invention in a sample taken from an individual,
b) detecting the level of expression of at least one of the proposed markers of the invention in a sample taken from a healthy individual,
c) comparing the difference in level of expression of at least one marker and for which the level of expression is

significantly higher in the individual than the level of expression in the healthy individual;
d) the overexpression of at least one of the markers of step c) being an indicator of acne, thus diagnosing acne.

[0009] The disclosure provides a method for monitoring the progression of acne, comprising the following steps:

a) taking a biological sample from the individual,
b) analysing the level of expression of at least one of the proposed markers, and/or at least one of the markers chosen from IL-6, IL-17A, IL-17F, IL-22, CCL20 in a sample taken and in which a variation in the expression of at least one of the markers is an indicator of the progression of acne. Progression of acne may be from a predominantly comedonal to a more inflammatory dominated state, it may also mean progression towards specific acne subtypes, like nodulocystic acne or acne conglobata for example. Progression might also occur in the other direction, from a more severe to a less severe form of acne.

[0010] The disclosure provides also a method for monitoring the efficacy of a treatment intended for treating acne, comprising the following steps:

a) administering the desired treatment to the individual identified as having one or more of the symptoms of acne,
b) taking a biological sample from the individual,
c) analysing the level of expression of at least one of the proposed markers of the invention and/or at least one of the markers chosen from IL-17A, IL-17F, IL-22, CCL20, in which a variation in the expression of at least one of the markers is an indicator in the treatment of acne.

[0011] The invention relates to an in vitro screening method of Th-17 cells differentiation inhibitors for treating acne, comprising determining the capacity of said candidate to inhibit or down regulate expression or biological activity of one of the proposed markers (RORgamma t or RORalpha) of the invention.

[0012] More specifically, the disclosure relates to an in vitro screening method of Th17 cells differentiation inhibitors for drug candidates, comprising the following steps:

a) Collecting at least two biological samples: one mimics the acne lesion, and one mimics the healthy condition;
b) Contacting at least one sample or a mixture of samples with one or more drug candidates to be tested;
c) Detecting the expression of at least one of the proposed markers, and/or at least one of the expression markers selected from: IL-17A, IL-17F, IL-22, CCL20 in the biological samples or mixture obtained in b);
d) Selecting drug candidates which are capable of inhibiting the expression of at least one of the proposed markers, and/or the expression of at least one of the expression markers selected from IL-17A, IL-17F, IL-22, CCL20 measured in said samples or mixtures obtained in b) and comparing the levels with a sample not mixed with the drug candidate(s).

[0013] In another embodiment, the disclosure provides an in vitro screening method of Th17 cells inhibitors for drug candidate, comprising the following steps:

a) Collecting at least two biological samples: one mimics the acne lesion, and one mimics the healthy condition;
b) Contacting at least one sample or a mixture of samples with one or more drug candidates to be tested;
c) Detecting the expression of at least one of the proposed markers in the biological samples or mixture obtained in step b);
d) Selecting drug candidates which are capable of inhibiting expression of at least one chosen from the proposed markers measured in said samples or mixture obtained in step b) and comparing the levels with a sample not mixed with the drug candidate.

[0014] Also disclosed is the use of inhibitors identified by screening methods as defined above for the preparation of a composition for treating acne and/or acne associated disorders. More specifically, the invention encompasses the use of inhibitors of the proposed markers identified by screening methods for the preparation of a composition for treating acne or acne associated disorders such as N-(2,2,2-trifluoroethyl)-N-[4-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]phenyl]-benzenesulfonamide, 2 oxysterol (oxygenated sterols), especially 24S-hydroxycholesterol 24(S), 25-epoxycholesterol and 7-oxygenated sterols, Methyl 2-cyano-3,12-dioxooleana-1,9(11)dien-28-oate or Bardoxolone methyl,-(8S,9R,10R,13R,14S,16R,17R)-17-[(E,2R)-2, 6-dihydroxy-6-methyl-3-oxohept-4-en-2-yl]-2,16-dihydroxy-4,4,9,13, 14-pentamethyl-8,10,12,15,16, 17-hexahydro-7H-cyclopenta[a]phenanthrene-3,11-dione, 5-(4-chlorophenyl)-6-ethylpyrimidine-2,4-diamine, gamma-D-glutamyl-L-tryptophan, 8-hydroxy-3-methyl-3,4-dihydro-2H-benzo[a]anthracene-1,7,12-trione, 5,7-dihydroxy-2-(4-hydroxyphenyl)-4-oxo-4H-chromen-3-olate, methyl-N-[4-(trifluoromethyl)phenyl]-1,2-oxazole-4-carboxamide or Leflunomide, N-[(E)-(3-methylphenyl)methylideneamino]-6-morpholin-4-yl-2-(2-pyri-

din-2-ylethoxy)pyrimidin-4-amine.

Detailed description

[0015] The invention is defined by the appended claims.

[0016] Indeed, Th17 cells, a distinct Th lineage originally from the differentiation of naive CD4+ T cells, provide immunity against a variety of extracellular pathogens, including bacteria and fungi. Interestingly, although P.acnes is a commensal bacteria present in healthy human skin, it has been regarded as one of the pathogenetic factors in acne vulgaris. However, it is still not clear whether P. Acnes is indeed a causal agent in the development of non-inflamed and inflamed acne lesions (Shaheen B, Gonzalez M. A microbial aetiology of acne-what is the evidence? Br J Dermatol. 2011 Apr 18).

[0017] Th17 cells have also been implicated in a variety of inflammatory and autoimmune disorders, such as psoriasis, rheumatoid arthritis and multiple sclerosis (Peck A, Mellins ED. Precarious balance: Th17 cells in host defense. Infect Immun. 2010 Jan;78(1):32-8).

[0018] At molecular level, Th17 cells are characterized by the production of a distinct profile of effector cytokines, IL-17A, IL-17F, IL-26, IL-22, IL-21 and TNFα and depend upon IL-23 for their development, survival and proliferation. These cytokines activate different type of cells, such as keratinocytes, leading to their hyperproliferation and further production of proinflammatory cytokines, chemokines and antimicrobial peptides, which in turn recruit and activate other immune cells in the inflamed skin, leading to amplification of the inflammatory response. Moreover, IL-17A, and IL-17F leading to an autocrine regulation of IL-17 production which serves to promote and sustain Th17 cells differentiation (Wei et al. 2007, J Biol. Chem., September 20). Il 17 is also responsible for the upregulation of CCL2O, the ligand of a characterized receptor of the TH17 cells in stromal cells, allowing the attraction of additional Th17 cells into inflamed tissue.

[0019] The signalling pathways of the naive CD4 T cell differentiation into Th17 cells required TGFb-1 either in combination with IL-21, with IL-1b and IL-23 or with IL-1b, IL-23, and IL-6, and lead to the expression of retinoid-related orphan receptor (RORC) and retinoid acid-related orphan receptor alpha (RORA), which are two transcription factors that promote TH17 differentiation and substantially upregulate IL-17A and IL-17F expression (Chung Y et al. Critical regulation of early Th17 cell differentiation by interleukin-1 signaling. Immunity 2009;30:576-87, Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. and Immunity 2006 Feb; 24(2):179-89).

[0020] For the following, "Th-17 differentiation profile molecules" refers to the biological molecules that characterize the Th17 cell differentiation that is to say the cytokines and/or factors of whom depends the differentiation from naive T cells to Th-17 cells, in other words IL-6, IL-26, IL-23 and/or which are produced by TH17 cells (IL-17A, IL-17F, IL-21, IL-22, IL-26, TNF alpha, CCL20), and/or also receptors expressed by TH17 cells (CCR6, IL-23R).

[0021] Animal experiments place mROR-yt (the mouse ortholog of human ROR gamma t) in the rank of a master regulator of Th-17 differentiation. ROR gamma t deficiency in mice results in diminished Th17 activity and severely reduced expression of IL-17 (Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, Cua DJ, Littman DR. The orphan nuclear receptor ROR gamma t directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell. 2006 Sep 22;126(6):1121-33).

[0022] The present disclosure provides ROR gamma t or ROR alpha, crucial actors of Th17 cell differentiation, as novel markers for characterizing acne with the examples which follow.

[0023] In particular embodiment, the disclosure provides the use of the mRNA encoding ROR gamma t and also the corresponding proteins, as markers for acne.

[0024] In particular embodiment, the disclosure provides the use of the mRNA encoding ROR alpha and also the corresponding proteins, as markers for acne.

[0025] In another embodiment, the disclosure provides the use of at least one of the proposed markers and/or at least one of the markers selected from the following list: IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-23 A, IL-26, TNF alpha or CCL20 as markers for acne.

[0026] In another embodiment, the disclosure provides the used of at least one of the proposed markers and/or at least one of the markers selected from the following list: IL-6, IL-17A, IL-17F, IL-22, CCL20, as markers for acne.

[0027] For the purpose of the present invention, the term "marker" or "biological marker" denotes a biological marker associated with the presence or with the absence of a particular pathological state. The biological markers are in particular proteins or mRNAs.

[0028] For more clarity, the following definitions are used: The term "Proposed markers" means ROR gamma t and/or ROR alpha. "ROR gamma t", means either the expression product of RORC variant 2, i.e ROR gamma mRNA or protein or the RORC gene itself. In analogy, "ROR alpha" means either the expression product of the RORA gene,i.e ROR alpha mRNA or protein or the RORA gene itself.

[0029] The term "level of expression" or "expression" means the level of mRNAs or proteins encoded by the gene marker.

[0030] The expression level analysis or detection can be performed by any suitable method, known to those skilled in the art, such as western blotting, IHC, mass spectrometry (Maldi-TOF and LC/MS analyses), radioimmunoassay (RIA),

Elisa or any other method known to those skilled in the art or else by assaying the mRNA according to the methods customarily known to those skilled in the art. The techniques based on the hybridization of mRNA with specific nucleotide probes are the most customary (Northern blotting, RT-PCR (Reverse Transcriptase Polymerase Chain Reaction), quantitative RT-PCR (qRT-PCR), RNase protection).

[0031] In one embodiment, the disclosure related to a method for the diagnosis of acne, comprises the following steps:

a) detecting the level of expression of at least one of the proposed above markers, and/or at least one of the markers chosen from IL-6, IL-17A, IL-17F, IL-22, CCL20 in a sample taken from an individual,
b) detecting the level of expression of and at least one of the above markers, and/or at least one of the markers chosen from IL-6, IL-17A, IL-17F, IL-22, CCL20 in a sample taken from a healthy individual,
c) comparing the difference in level of expression of at least one marker and for which the level of expression is significantly higher in the sample taken from the individual than the level of expression in the healthy individual;
d) the overexpression of at least one of the markers of step c) being an indicator of acne, thus diagnosing acne.

[0032] The method for the diagnosis of acne can also comprise the following steps:

a) detecting the level of expression of at least one of the proposed markers in a sample taken from an individual,
b) detecting the level of expression of at least one of the proposed markers in a sample taken from a normal individual,
c) comparing the difference in level of expression of at least one marker and for which the level of expression is significantly higher in the sample of the individual than the level of expression in the healthy individual;
d) the overexpression of at least one of the markers of step c) being an indicator of acne, thus diagnosing acne.

[0033] According to another aspect, the disclosure is related to a method for monitoring the progression or variation of acne, comprising the following steps:

a) taking a biological sample from the individual,
b) analysing the level of expression of at least one of the proposed markers, and/or at least one of the markers chosen from IL-6, IL-17A, IL-17F, IL-22, CCL20 in a sample taken and in which a variation in the expression of at least one of the markers is an indicator of the progression of acne. Thus, the invention relates also to a method for the prognosis of the progression or variation of acne.

[0034] According to another aspect the disclosure is related to a method for monitoring the efficacy of a treatment intended for treating acne, comprising the following steps:

a) administering the desired treatment to the individual identified as having one or more of the symptoms of acne,
b) taking a biological sample from the individual,
c) analysing the level of expression of at least one of the proposed markers and/or one of the other markers chosen from I16, IL-17A, IL-17F, IL-22, CCL20, in the sample taken in b), according to any suitable technique known to those skilled in the art, in which a variation in the expression of at least one of the markers is an indicator in the treatment of acne.

[0035] Preferably, the expression of at least one of the above mentioned markers decreases or moves closer to the level of expression known for a healthy individual.

[0036] The expression "overexpression of one of the factors or markers" is intended to mean a level of expression increased by at least 50%, and preferably by at least 100%, and even more preferably by at least 200%, or expressed differently with equivalent significance, by at least a factor of 2, or at least twice as high as the level in a normal individual; which demonstrates overall an overexpression of the chemokines, the cytokines and the receptors mentioned above, thus representing markers characteristic of acne.

[0037] In the context of the invention, the biological sample corresponds to any type of sample taken from an individual, and can be a tissue sample or a fluid sample, such as blood, lymph or interstitial fluid.

[0038] According to one particular and preferred embodiment, the sample is a biopsy of varying size (preferably from 1 to 6 mm in diameter), or a skin sample taken by means of tape stripping, such as with D-Squames, according to the method described in Wong R et al., "Analysis of RNA recovery and gene expression in the epidermis using non-invasive tape stripping"; J Dermatol Sci.2006 Nov; 44(2):81-92; or in Benson NR, et al., "An analysis of select pathogenic messages in lesional and non-lesional psoriatic skin using non-invasive tape harvesting". J Invest Dermatol. 2006 Oct; 126(10): 2234-41; or else in Wong R et al., "Use of RT-PCR and DNA microarrays to characterize RNA recovered by non-invasive tape harvesting of normal and inflamed skin". J Invest Dermatol. 2004 Jul; 123(1):159-67. According to the principle of tape stripping, the product used comprises a flexible translucent polymer support and an adhesive. The product is applied

repeatedly to the skin of the patient, preferably until loss of adhesion. The sample obtained relates only to the content of the outermost layers of the epidermis. A method for analysing a protein content obtained in particular according to this sampling method is described in Patent Application WO2009/068825 (Galderma R&D) in order to monitor markers specific for a pathological skin condition and to orient the diagnosis. Since this method is rapid, non-invasive and relatively inexpensive for detecting the presence of, the absence of or the variation in certain proteomic markers, it is particularly preferred. This method is in particular characterized by mass spectrometry detection, ELISA or any other method known to the expert skilled in the art of protein quantification. Quantification is performed in the skin sample obtained on the flexible and adhesive support in order to detect at least one protein of which the presence, the absence or the variation in amount or in concentration compared with a standard value is associated with the presence, with the progression or with the absence of a particular pathological skin condition.

[0039] Another embodiment of the present disclosure is an in vitro screening method of Th17 cell differentiation candidate inhibitors, comprising determining the capacity of said candidate to inhibit and/or down regulate the expression or the biological activity or the biological function, including the transactivation properties, of at least one of the proposed markers (RORgamma t or RORalpha) of the disclosure.

[0040] The identified candidate will influence the biological function of a given marker or a biological process modulated by the marker. For example, the inhibition of ROR gamma t and/or ROR alpha by a candidate may affect the biological function of ROR gamma t, including the induction of the Th17 cell differentiation as well as the function of Th17 cells.

[0041] For screening purposes, the biological samples consist of transfected cells containing reporter genes operating under the control of a promoter (totally or partially) controlling the expression of an above mentioned gene. Alternatively, the promoter may be, at least in part, synthetically assembled and contain ROR-responsive elements. The ability of a compound to modulate the function of the proposed markers, is evaluated by analyzing the expression of the reporter gene.

[0042] The transfected cells may further be engineered to express at least one of the proposed markers.

[0043] The reporter gene may encode an enzyme that with its corresponding substrate, provides coloured product(s) such as CAT (chloramphenicol acetyltransferase), GAL (beta galactosidase), or GUS (beta glucuronidase). It might be either luciferase or GFP (Green Fluorescent Protein).

[0044] Reporter gene protein dosage or its activity is typically assessed by colourimetric, fluorometric or chemoluminescence methods.

[0045] According to a further embodiment of the disclosure, biological samples are cells expressing the gene of interest and the step c) above consists to measure the activity of the gene product.

[0046] Also disclosed is to the use of identified inhibitors/antagonists/inverse agonists with the described screening methods for the preparation of a composition for treating acne and/or acne associated disorders.

[0047] In particular, the inhibitors/antagonists/inverse agonists of ROR gamma t or ROR alpha could be selected from the following list:

- N-(2,2,2-trifluoroethyl)-N-[4-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]phenyl]-benzenesulfonamide; this compound is a novel retinoic acid receptor-related orphan receptor-alpha/gamma inverse agonist. (Mol Pharmacol. 2010 Feb;77(2):228-36))
- 2 oxysterol (oxygenated sterols), especially 24S-hydroxycholesterol 24(S), 25-epoxycholesterol and 7-oxygenated sterols [a second class of nuclear receptors for oxysterols: Regulation of RORalpha and RORgamma activity by 24S-hydroxycholesterol (cerebrosterol)- Wang Y et al. Biochim Biophys Acta. 2010 Aug; 1801(8):917-23. Epub 2010 Mar 6]; Wang Y et al. Modulation of retinoic acid receptor-related orphan receptor alpha and gamma activity by 7-oxygenated sterol ligands. J Biol Chem. 2010 Feb 12; 285(7):5013-25))
- Methyl2-cyano-3,12-dioxooleana-1,9(11)dien-28-oate or Bardoxolone methyl (also known as "RTA 402" and "CDDO-methyl ester).
- (8S,9R,10R,13R,14S,16R,17R)-17-[(E,2R)-2, 6-dihydroxy-6-methyl-3-oxohept-4-en-2-yl]-2,16-dihydroxy-4,4,9,13, 14-pentamethyl-8,10,12,15,16, 17-hexahydro-7H-cyclopenta[a]phenanthrene-3,11-dione or SI-124 (Blaskovich MA, Sun J, Cantor A et al.Discovery of JS-124 (cucurbitacin I), a selective Janus Kinase/ Signal Transducer and Activator of Transcription 2 signaling pathway inhibitor with potent antitumor activity against human and murine cancer cells in mice Cancer Res 2003; 63: 1270-1279)
- Pyrimethamine: - 5-(4-chlorophenyl)-6-ethylpyrimidine-2,4-diamine or Pyrimethamine (Dariprim)(WO/2008/156644)
- gamma-D-glutamyl-L-tryptophan or SCV-07 (SciClone Pharmaceuticals)(Nagabhushanam V, Subbarao K, Ramachandran M et al Inhibition of STAT3 driven gene expression in melanoma cells by SCV-07 J Clin Oncol 2008; 26 (May 20, suppl): 14619)
- 8-hydroxy-3-methyl-3,4-dihydro-2H-benzo[a]anthracene-1,7,12-trione or STA-21 (Song H, Wang R, Wang S et al.A low-molecular-weight compound discovered through virtual database screening inhibits Stat3 function in breast cancer cells PNAS 2005; 102: 4700-4705
- natural flavonol: such as 5,7-dihydroxy-2-(4-hydroxyphenyl)-4-oxo-4H-chromen-3-olate or Kaempferol (Bruno RD,

Njar VC. Targeting cytochrome P450 enzymes: a new approach in anti-cancer drug development. Bioorg Med Chem. 2007 Aug 1;15(15):5047-60. Epub 2007 May 23).

- methyl-N-[4-(trifluoromethyl)phenyl]-1,2-oxazole-4-carboxamide or Leflunomide (O'Donnell EF, Saili KS, Koch DC, Kopparapu PR, Farrer D, Bisson WH, Mathew LK, Sengupta S, Kerkvliet NI, Tanguay RL, Kolluri SK. The anti-inflammatory drug leflunomide is an agonist of the aryl hydrocarbon receptor. PLoS One. 2010 Oct 1;5(10).
- N-[(E)-(3-methylphenyl)methylideneamino]-6-morpholin-4-yl-2-(2-pyridin-2-ylethoxy)pyrimidin-4-amine or STA 5326 (Apilimod Synta pharmaceuticals) Wada et al: Selective abrogation of Th1 response by STA-5326, a potent IL-12/IL-23 inhibitor. Blood, 2007, 109(3), 1156-1164. ; Wada et al: IL-12/IL-23 inhibitors: a promising approach to the treatment of inflammatory disorders. Drugs Fut. 2008, 33(1), 49-63
- [(3S,5R,8R,9S,10S,12R,13S,14S)-3-[(2S,4S,5R,6R)-5-[(2S,4S,5R,6R)-5-[(2S,4S,5R,6R)-4,5-dihydroxy-6-methyl-oxan-2-yl]oxy-4-hydroxy-6-methyl-oxan-2-yl]oxy-4-hydroxy-6-methyl-oxan-2-yl]oxy-12,14-dihydroxy-10,13-dimethyl-1, 2,3,4,5,6,7,8,9,11,12,15,16,17-tetra decahydrocyclopenta[a]phenanthren-17-yl]-5H-furan-2-one or Digoxin and its derivatives.

[0048] In another aspect, inhibitors might be either a polypeptide, a DNA or an antisense RNA, an si-RNA or a PNA ("Peptide nucleic acid", i-e with a polypeptidic chain substituted by purine and pyrimidine bases and having a DNA-like structure for hybridization to this latter).

[0049] The modulator might be an antibody and preferably a monoclonal antibody. Advantageously, the monoclonal antibody is administered to a patient in a sufficient quantity so as the measure a plasmatic concentration is from about 0.01μg/ml to about 100μg/ml, preferred from about 1μg/ml to about 5μg/ml.

[0050] The invention is intended for treating acne. By acne it is understood, all acne forms especially simple acne, comedonic acne, papulopustular acne, papulocomedonic acne, nodulocystic acne, acne conglobata, cheloid acne of the nape of the neck, recurrent miliary acne, necrotic acne, neonatal acne, occupational acne, acne rosacea, senile acne, solar acne and medication-related acne and also more largely, acne associated disorders (e.g. hyperseborrhoea).

[0051] The example which follows illustrates the invention without limiting the scope thereof.

Table 1: mRNA expression measured by Affymetrix Technology. Analysis of Th17 differentiation profile molecules IL-17A, IL-17F, IL-26, IL-6 and proposed markers RORA and RORC, as well as IL-5, IL-4 IL-13 typically considered as Th2 cytokines.

Table 2: mRNA expression measured by qRT-PCR (TaqMan low density Array technology). Analysis of the expression of Th17 differentiation profile molecules IL-6, IL-17, IL-22, IL-23, CCL20, IL-6 and RORC, one of the proposed markers, as well as IL-5, IL-4 and IL-13 typically considered as Th2 cytokines.

Table 3: Protein expression of Th17 differentiation profile molecules (Luminex assay). Analysis of IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-23a, CCL20 and TNF alpha, as well as IL-5, IL-4 and IL-13 typically considered as Th2 cytokines.

FIGURE 1: Acne lesion: T lymphocyte immunohistochemical detection (CD3 alone)

FIGURE 2 : Acne lesion : IL-17 expression in T lymphocytes (CD3/IL 17 co-localisation)

EXAMPLE 1: Modulation of the TH17 molecular profile in the lesional skin of patients suffering from acne compared with non-lesional skin of these patients: Analysis of the expression of IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-23A, IL-26, TNF alpha, CCL20 and the proposed markers RORA and RORC

Patient selection and tissue biopsies:

[0052] Skin biopsies of acne patients were obtained from an inflammatory papule and from non lesional skin in 12 patients with acne, in accordance with good clinical practice. (The clinical description of acne subtypes was carried out according to the classification of Wilkin et al., 2002, J. Am. Acad. Dermatol. Vol 46, pages 584-587.)

[0053] To evaluate a change in the expression level of the genes, the expression levels in lesional skin are compared with the expression levels in non-lesional skin of the same subjects (n=12).

mRNA extraction, labelling and hybridization to probe arrays :

[0054] The mRNA was isolated from skin using the RNeasy extraction kit (Quigen Inc., Valencia,CA) and quality was evaluated using a 2100 Bioanalyser of Agilent. The mRNA expression was evaluated by a Gene Chip IVT labelling kit after the generation of double-stranded cDNA (i.e in vitro transcription process) using T7-oligo primer and the one cycle

cDNA synthesis kit of Affymetrix. RNA was ethanol precipitated to concentrate the sample and then quantified using a spectrophotometer. Approximately 200 ng of total RNA of good quality [RNA indication number (RIN) ≥ 7] from each sample was used to generate double-stranded cDNA using a T7-oligo (dt) primer (one cycle cDNA synthesis kit, Affymetrix). Biotinylated cRNA, produced through in vitro transcription (Gene Chip IVT labelling kit, Affymetrix) was fragmented and hybridised to an Affymetrix human U133A 2.0 plus microarray. The arrays were processed on a Gene Chip Fluidics Station 450 and scanned on an Affymetrix Gene Chip Scanner (Santa Clara, CA) .

Statistical Analysis of mRNA expression based on Affymetrix gene chips:

[0055] The expression data from Affymetrix Gene Chips are normalized with RMA (Robust Multi-array Analysis) method. The raw intensity values are background corrected, log2 transformed and then quantile normalized. Next a linear model is fit to the normalized data to obtain an expression measure for each probe set on each array. To identify genes that were significantly modulated in the different Acne subtype samples, one-way ANOVA with Benjamini-Hochberg multiplicity correction was performed using JMP 7.0.1 (SAS Institute) and irMF 3.5 (National Institute of Statistical Sciences, NISS) software.

qRT-PCR Measurement of mRNA expression:

[0056] The expression of the Th17 differentiation profile was also measured by qRT-PCR.

[0057] In the following table the expression levels are documented using the Mean Ct (Cycle Threshold) of individual genes in non lesional skin and in lesional skin of acne patients. The Ct value is inversely proportional to the quantity of the mRNA of a given gene.

Cytokine extraction and assay:

[0058] Proteins were extracted from inflammatory papules and non lesional skin in 12 patients with acne. Cytokines were dosed in the protein extracts using Luminex assays (Millipore & Procarta cytokine dosage kits). The cytokine quantities were normalized to the total concentration of protein. Paired P-values were calculated for each cytokine.

[0059] The mRNA expression of the Th17 differentiation profile molecules: IL-17A, IL-17F, IL-26, CCL20 and proposed markers ROR A and ROR C were measured using Affymetrix technology (Table 1) and qRT-PCR (Table 2).

[0060] The mRNA of specific cytokines IL-17A, IL-17F, IL-22, IL-26 characterizing Th17 cells differentiation measured by Affymetrix (table 1) or qRT-PCR (table 2) techniques are significantly up-regulated in lesional skin. Moreover, in these tables, the mRNA expression of IL5, IL4 and IL13 are not detected or not changed suggesting that the inflammatory response in acne is not driven by Th2 cells.

[0061] Table 3 demonstrates an up-regulation of the protein expression level of IL-6, IL-17A, IL-17F, IL-21, IL-22 and TNF alpha in lesional skin in comparison to non-lesional skin.

[0062] Thus, these expression differences of cytokines implicated in the Th-17 cell differentiation process demonstrate the interest of inhibiting or targeting Th17 cell differentiation for the treatment or diagnosis of acne.

[0063] Surprisingly, the mRNA levels of the transcription factors ROR A and ROR C are slightly decreased in acne, but their expression in human skin was clearly demonstrated. Thus, they are interesting as markers for diagnosing acne and/or screening inhibitors of Th17 cells differentiation. For diagnostic purposes, they can be used alone or in combination with at least one of the Th17 cell differentiation profile molecules mentioned previously.

EXAMPLE 2: Immunohistochemistry analysis:

[0064] In normal skin, we observed hardly any lymphocyte infiltrates whereas in biopsies from lesional areas there were found in greater numbers. In this context, IL17 detection using an immunohistochemistry technique was performed in these infiltrates from lesional areas.

[0065] A first primary antibody (anti CD3) was used in order to detect the T lymphocytes, followed by a second antibody, specific for IL-17.

[0066] These antibodies were respectively revealed with a second antibody combined with a red fluorophore (TRITC) or a green fluorophore (FITC).

[0067] The results for CD3 expression are presented in Figure 1. The positive cells, in black, confirmed that the infiltrate was largely composed of T lymphocytes.

[0068] Figure 2 demonstrates that a subpopulation of CD3 positive T lymphocytes co-expressed IL-17. This positive IL-17 staining suggests the presence of Th17 cells in acne lesions.

Table 1

GENE_ SYMBOL	TITLE	Non lesional skin Mean_ Expressions	Lesional skin Mean_ Expressions	Lesional skin vs non lesional skin Fold Change	Lesional skin vs non lesional skin Adjusted Pvalue
IL6	interleukin 6 (interferon, beta 2)	**73**	**340**	**4,7**	**1,3E-03**
IL17A	interleukin 17A	**13**	**57**	**4,3**	**7,8E-03**
CCL20	chemokine (C-C motif) ligand 20	**146**	**482**	**3,3**	**1,6E-03**
IL17F	interleukin 17F	**35**	**88**	**2,5**	**1,1E-02**
IL26	interleukin 26	**24**	**48**	**2,0**	**2,3E-03**
RORA	RAR-related orphan receptor A	**9776**	**4542**	**-2,2**	**6,0E-04**
RORC	RAR-related orphan receptor C	**236**	**106**	**-2,2**	**7,0E-04**
IL5	interleukin 5 (colony-stimulating factor, eosinophil)	**Not detected**	**Not detected**		
IL4	interleukin 4	**Not detected**	**Not detected**		
IL13	interleukin 13	**78,61**	**79,65**	**1,0**	**8,0E-01**

Table 2

GENE_ SYMBOL	TITLE	Non lesional skin Mean_ CT	Lesional skin Mean_ CT	Lesional skin vs non lesional skin Fold Change	Lesional skin vs non lesional skin P-value
IL23A	**interleukin 23, alpha subunit p19**	**32,8**	**30,1**	**6,23**	**1,5E-08**
IL6	**interleukin 6 (interferon, beta 2)**	**30,3**	**27,7**	**6,22**	**<0.0001**
IL17A	**interleukin 17A**	**32,3**	**29,9**	**5,55**	**<0.0001**
IL22	**interleukin 22**	**34,0**	**32,7**	**2,42**	**8,1E-06**
CCL20	**chemokine (C-C motif) ligand 20**	**29,2**	**28,0**	**2,35**	**6,8E-10**
RORC	**RAR-related orphan receptor C**	**24,3**	**26,8**	**-5,47**	**<0.0001**
IL5	**interleukin 5 (colony-stimulating factor, eosino**	**34,3**	**35,4**	**-2,08**	**5,26E-13**
IL4	**interleukin 4**	**34,2**	**35,3**	**-2,12**	**2,03E-08**
IL13	**interleukin 13**	**34,3**	**35,4**	**-2,19**	**2,53E-13**

Table 3

Proteins	LSP	NSP	FoldChange	PValue
Interleukin-6	16,2	1,1	15,0	0,0005
interleukin-17F	1,2	0,3	4,9	3,2E-03
Interleukin-21	29,1	7,5	3,9	1,1E-02
Interleukin-17A	4,0	1,1	3,8	0,0081
Interleukin-22	14,3	4,6	3,1	3,2E-02
Interleukin-23 subunit alpha	1,0	0,3	3,1	1,7E-01

(continued)

Proteins	LSP	NSP	FoldChange	PValue
C-C motif chemokine 20	1,0	0,4	2,6	4,0E-02
Tumor necrosis factor alpha	2,6	1,1	2,5	0,0182
Interleukin-4	4,2	1,9	2,2	0,0052
Interleukin-5	not detected	not detected		
Interleukin-13	3,3	2,1	1,6	0,0678

Claims

1. Use of (i) the mRNA encoding ROR gamma t, or the corresponding ROR gamma t protein, and (ii) the mRNA encoding at least one marker selected from IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-23A, IL-26 and CCL20, or the corresponding protein, as markers for acne.

2. Use of (i) the mRNA encoding ROR alpha, or the corresponding ROR alpha protein, and (ii) the mRNA encoding at least one marker selected from IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-23A, IL-26 and CCL20, or the corresponding protein, as markers for acne.

3. A method for the diagnosis of acne, comprising the following steps of:

   a) detecting the level of expression of (i) the mRNA encoding ROR gamma t, or the corresponding protein, or (ii) the mRNA encoding ROR alpha, or the corresponding protein, and (iii) the mRNA encoding at least one of the markers selected from IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-23A, IL-26 and CCL20, or the corresponding protein, in a sample from an individual,
   b) detecting the level of expression of (i) the mRNA encoding ROR gamma t, or the corresponding protein, or (ii) the mRNA encoding ROR alpha, or the corresponding protein, and (iii) the mRNA encoding at least one of the markers selected from IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-23A, IL-26 and CCL20, or the corresponding protein, in a sample of non lesional skin, optionally from a healthy individual,
   c) comparing the levels of expression of the mRNA encoding the markers, or the corresponding proteins, respectively detected in step b) and in step a), the variation in the expression of mRNA encoding the markers, or the corresponding proteins, being an indicator of acne, thus diagnosing acne.

4. A method for monitoring the progression of acne in an individual, comprising the step of analysing the level of expression of (i) the mRNA encoding ROR gamma t, or the corresponding protein, or (ii) the mRNA encoding ROR alpha, or the corresponding protein, and (iii) the mRNA encoding at least one marker selected from IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-23A, IL-26 and CCL20, or the corresponding protein, in a biological sample wherein a variation in the levels of expression of the mRNA encoding at least one of the markers, or the corresponding protein, at different time is an indicator of the progression of acne.

5. A method for monitoring the efficacy of a treatment for treating acne in an individual, comprising the step of analysing the level of expression of (i) the mRNA encoding ROR gamma t, or the corresponding protein, or (ii) the mRNA encoding ROR alpha, or the corresponding protein, and (iii) the mRNA encoding at least one marker selected from IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-23A, IL-26, and CCL20, or the corresponding protein, in a biological sample obtained from an individual identified as having one or more of the symptoms of acne, wherein a variation in the expression of mRNA encoding at least one of the markers, or the corresponding protein, is an indicator of efficacy in the treatment of acne.

6. An *in vitro* screening method of Th17 cells differentiation inhibitors for treating acne, comprising determining the capacity of a candidate to inhibit or down regulate expression of (i) the mRNA encoding ROR gamma t, or the corresponding protein, or (ii) the mRNA encoding ROR alpha, or the corresponding protein, and (iii) the mRNA encoding at least one marker selected from IL-6, IL-17A, IL-17F, IL21, IL-22, IL-23A, IL-26, and CCL20, or the corresponding protein.

7. An *in vitro* screening method of Th17 cells differentiation inhibitors for the identification of drug candidates for treating acne, comprising the following steps of:

a) contacting at least one biological sample mimicking an acne lesion or a mixture of biological samples mimicking an acne lesion with one or more drug candidates to be tested;
b) detecting the expression of (i) the mRNA encoding ROR gamma t, or the corresponding protein, or (ii) the mRNA encoding ROR alpha, or the corresponding protein, and (iii) the mRNA encoding at least one marker selected from IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-23A, IL-26, and CCL20, or the corresponding protein, in the biological sample or mixture of step a); and
c) selecting drug candidates which inhibit or down regulate the expression of (i) the mRNA encoding ROR gamma t, or the corresponding protein, or (ii) the mRNA encoding ROR alpha, or the corresponding protein, and (iii) the mRNA encoding at least one marker selected from IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-23A, IL-26, and CCL20, or the corresponding protein, in the biological sample or mixture of step a), as detected in step b), said selected candidates being identified as the Thl7 cells differentiation inhibitors.

Patentansprüche

1. Verwendung (i) der für ROR gamma t kodierenden mRNA oder des entsprechenden ROR gamma t-Proteins und (ii) der für mindestens einen Marker ausgewählt aus IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-23A, IL-26 und CCL20 kodierenden mRNA oder des entsprechenden Proteins als Marker für Akne.

2. Verwendung (i) der für ROR alpha kodierenden mRNA oder des entsprechenden ROR alpha-Proteins und (ii) der für mindestens einen Marker ausgewählt aus IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-23A, IL-26 und CCL20 kodierenden mRNA oder des entsprechenden Proteins als Marker für Akne.

3. Verfahren zur Diagnose von Akne, bei dem man

a) das Expressionsniveau (i) der für ROR gamma t kodierenden mRNA oder des entsprechenden Proteins, oder (ii) der für ROR alpha kodierenden mRNA oder des entsprechenden Proteins, und (iii) der für mindestens einen Marker ausgewählt aus IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-23A, IL-26 und CCL20 kodierenden mRNA oder des entsprechenden Proteins in einer Probe eines Individuums feststellt,
b) das Expressionsniveau (i) der für ROR gamma t kodierenden mRNA oder des entsprechenden Proteins, oder (ii) der für ROR alpha kodierenden mRNA oder des entsprechenden Proteins, und (iii) der für mindestens einen Marker ausgewählt aus IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-23A, IL-26 und CCL20 kodierenden mRNA oder des entsprechenden Proteins in einer Probe von nicht-läsionaler Haut, wahlweise von einer gesunden Person, feststellt,
c) die Expressionsniveaus der für die Marker kodierenden mRNA oder der entsprechenden Proteine, die in Schritt (b) bzw. in Schritt (a) festgestellt wurden, vergleicht, wobei die Variation in der Expression der für die Marker kodierenden mRNA oder der entsprechenden Proteine ein Indikator für Akne ist.

4. Verfahren zur Überwachung des Fortschreitens von Akne in einem Individuum, umfassend den Schritt der Analyse des Expressionsniveaus (i) der für ROR gamma t kodierenden mRNA oder des entsprechenden Proteins oder (ii) der für ROR alpha kodierenden mRNA oder des entsprechenden Proteins, und (iii) der für mindestens einen Marker ausgewählt aus IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-23A, IL-26 und CCL20 kodierenden mRNA oder des entsprechenden Proteins in einer biologischen Probe, wobei eine Variation in den Expressionsniveaus der für mindestens einen der Marker kodierenden mRNA oder des entsprechenden Proteins zu unterschiedlichen Zeitpunkten ein Indikator für das Fortschreiten der Akne ist.

5. Verfahren zum Überwachen des Wirksamkeit einer Behandlung zur Behandlung von Akne in einem Individuum bei einer Person, umfassend den Schritt der Analyse des Expressionsniveaus (i) der für ROR gamma t kodierenden mRNA oder des entsprechenden Proteins oder (ii) der für ROR alpha kodierenden mRNA oder des entsprechenden Proteins, und (iii) der für mindestens einen Marker ausgewählt aus IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-23A, IL-26 und CCL20 kodierenden mRNA oder des entsprechenden Proteins in einer biologischen Probe, die aus einem Individuum erhalten wurde, welches als ein solches identifiziert wurde, das ein oder mehrere Symptome von Akne zeigt, wobei eine Variation in der Expression der für mindestens einen der Marker kodierenden mRNA oder des entsprechenden Proteins ein Indikator für die Wirksamkeit der Behandlung der Akne ist.

6. In vitro-Verfahren zum Screening von Inhibitoren der Thl7 Zelldifferenzierung zur Behandlung von Akne, bei dem man die Fähigkeit eines Kandidaten feststellt, die Expression (i) der für ROR gamma t kodierenden mRNA oder des entsprechenden Proteins oder (ii) der für ROR alpha kodierenden mRNA oder des entsprechenden Proteins,

und (iii) der für mindestens einen Marker ausgewählt aus IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-23A, IL-26 und CCL20 kodierenden mRNA oder des entsprechenden Proteins zu inhibieren oder herunter zu regulieren.

7. In vitro-Verfahren zum Screening von Inhibitoren der Thl7 Zelldifferenzierung zur Identifizierung von Wirkstoff-Kandidaten zur Behandlung von Akne, bei dem man

(a) mindestens eine biologische Probe, die eine Akne-Läsion nachahmt oder eine Mischung aus biologischen Proben, die eine Akne-Läsion nachahmen, mit einem oder mehreren zu untersuchenden Wirkstoff-Kandidaten in Kontakt bringt;
(b) die Expression (i) der für ROR gamma t kodierenden mRNA oder des entsprechenden Proteins oder (ii) der für ROR alpha kodierenden mRNA oder des entsprechenden Proteins, und (iii) der für mindestens einen Marker ausgewählt aus IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-23A, IL-26 und CCL20 kodierenden mRNA oder des entsprechenden Proteins in der biologischen Probe oder der Mischung von Schritt (a) feststellt; und
(c) Wirkstoff-Kandidaten auswählt, die die Expression (i) der für ROR gamma t kodierenden mRNA oder des entsprechenden Proteins oder (ii) der für ROR alpha kodierenden mRNA oder des entsprechenden Proteins, und (iii) der für mindestens einen Marker ausgewählt aus IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-23A, IL-26 und CCL20 kodierenden mRNA oder des entsprechenden Proteins in der biologischen Probe oder der Mischung von Schritt (a) inhibiert oder herunter reguliert, wie es in Schritt (b) festgestellt wird, wobei die ausgewählten Kandidaten als Inhibitoren der Th17-Zelldifferenzierung identifiziert werden.

Revendications

1. Utilisation de (i) l'ARNm codant pour ROR gamma t ou de la protéine ROR gamma t correspondante, et (ii) de l'ARNm codant pour au moins un marqueur choisi parmi IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-23A, IL-26 et CCL20, ou de la protéine correspondante, en tant que marqueurs de l'acné.

2. Utilisation de (i) l'ARNm codant pour ROR alpha ou de la protéine ROR alpha correspondante, et (ii) de l'ARNm codant pour au moins un marqueur choisi parmi IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-23A, IL-26 et CCL20, ou de la protéine correspondante, en tant que marqueurs de l'acné.

3. Méthode pour le diagnostic de l'acné, comprenant les étapes suivantes :

a) détecter le niveau d'expression de (i) l'ARNm codant pour ROR gamma t, ou de la protéine correspondante, ou (ii) de l'ARNm codant pour ROR alpha, ou de la protéine correspondante, et (iii) de l'ARNm codant pour au moins l'un des marqueurs choisis parmi IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-23A, IL-26 et CCL20, ou de la protéine correspondante, dans un échantillon d'un individu,
b) détecter le niveau d'expression (i) de l'ARNm codant pour ROR gamma t, ou de la protéine correspondante, ou (ii) de l'ARNm codant pour ROR alpha, ou de la protéine correspondante, et (iii) de l'ARNm codant pour au moins l'un des marqueurs choisis parmi IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-23A, IL-26 et CCL20, ou de la protéine correspondante, dans un échantillon de peau sans lésion, facultativement provenant d'un individu sain,
c) comparer les niveaux d'expression de l'ARNm codant pour les marqueurs, ou des protéines correspondantes, respectivement détectés à l'étape b) et à l'étape a), la variation de l'expression de l'ARNm codant pour les marqueurs, ou des protéines correspondantes, étant un indicateur de l'acné, en diagnostiquant ainsi l'acné.

4. Méthode de surveillance de la progression de l'acné chez un individu, comprenant l'étape consistant à analyser le niveau d'expression (i) de l'ARNm codant pour ROR gamma t, ou de la protéine correspondante, ou (ii) de l'ARNm codant pour ROR alpha, ou de la protéine correspondante, et (iii) de l'ARNm codant pour au moins un marqueur choisi parmi IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-23A, IL-26 et CCL20, ou de la protéine correspondante, dans un échantillon biologique, dans laquelle une variation des niveaux d'expression de l'ARNm codant pour au moins l'un des marqueurs, ou de la protéine correspondante, à un moment différent est un indicateur de la progression de l'acné.

5. Méthode de surveillance de l'efficacité d'un traitement pour traiter l'acné chez un individu, comprenant l'étape consistant à analyser le niveau d'expression (i) de l'ARNm codant pour ROR gamma t, ou de la protéine correspondante, ou (ii) de l'ARNm codant pour ROR alpha, ou de la protéine correspondante, et (iii) de l'ARNm codant pour au moins un marqueur choisi parmi IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-23A, IL-26 et CCL20, ou de la protéine correspondante, dans un échantillon biologique obtenu auprès d'un individu identifié comme présentant un ou plusieurs des symptômes de l'acné, dans laquelle une variation de l'expression de l'ARNm codant pour au moins

l'un des marqueurs, ou de la protéine correspondante, est une indicateur d'efficacité dans le traitement de l'acné.

6. Méthode de criblage *in vitro* d'inhibiteurs de différenciation de cellules Th17 pour le traitement de l'acné, comprenant la détermination de la capacité d'un candidat à inhiber ou à réguler à la baisse l'expression (i) de l'ARNm codant pour ROR gamma t, ou de la protéine correspondante, ou (ii) de l'ARNm codant pour ROR alpha, ou de la protéine correspondante, et (iii) de l'ARNm codant pour au moins un marqueur choisi parmi IL-6, IL-17A, IL-17F, IL21, IL-22, IL-23A, IL-26 et CCL20, ou de la protéine correspondante.

7. Méthode de criblage *in vitro* d'inhibiteurs de différenciation de cellules Th17 pour l'identification de médicaments candidats pour le traitement de l'acné, comprenant les étapes suivantes consistant à :

a) mettre en contact au moins un échantillon biologique imitant une lésion d'acné ou un mélange d'échantillons biologiques imitant une lésion d'acné avec un ou plusieurs médicaments candidats à tester ;
b) détecter l'expression (i) de l'ARNm codant pour ROR gamma t, ou de la protéine correspondante, ou (ii) de l'ARNm codant pour ROR alpha, ou de la protéine correspondante, et (iii) de l'ARNm codant pour au moins un marqueur choisi parmi IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-23A, IL-26 et CCL20, ou de la protéine correspondante, dans l'échantillon biologique ou le mélange de l'étape a) ; et
c) sélectionner des médicaments candidats qui inhibent ou régulent à la baisse l'expression (i) de l'ARNm codant pour ROR gamma t, ou de la protéine correspondante, ou (ii) de l'ARNm codant pour ROR alpha, ou de la protéine correspondante, et (iii) de l'ARNm codant pour au moins un marqueur choisi parmi IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-23A, IL-26 et CCL20, ou de la protéine correspondante, dans l'échantillon biologique ou le mélange de l'étape a), comme détecté à l'étape b), lesdits candidats sélectionnés étant identifiés en tant qu'inhibiteurs de différenciation de cellules Th17.

Figure 1

Figure 2

REFERENCES CITED IN THE DESCRIPTION

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